SHOCK, Vol. 34, No. 4, pp. 364–368, 2010

ENHANCED ALBUMIN SYNTHESIS IN SEVERELY BURNED ADULTS

Wenjun Z. Martini,* Steven E. Wolf,* David L. Chinkes,† Kevin K. Chung,* Michael A. Dubick,* Lorne Blackbourne,* and Yong-Ming Yu‡

**US Army Institute of Surgical Research, Ft Sam, Houston; †University of Texas Medical Branch, Galveston, Texas; and ‡Harvard Medical School and Massachusetts General Hospital, Boston, Massachusetts*

Received 20 Jan 2010; first review completed 8 Feb 2010; accepted in final form 9 Feb 2010

ABSTRACT—Albumin plays an important role in maintaining physiological homeostasis. Although decreased albumin concentration has been well described as an acute-phase response following injury, it is unclear whether the decrease is due to compromised synthesis of albumin, dilution, or imbalance between synthesis and breakdown rates, particularly after injury. We investigated changes in albumin synthesis in severely burned patients using stable isotope infusion techniques. Five patients (29 ± 3 years; 80 ± 7 kg) with burn of 48% ± 4% total body surface area (TBSA) were enrolled and studied in the ICU at the Burn Unit of the US Army Institute of Surgical Research. Five age- and sex-matched healthy volunteers (33 ± 5 years; 81 ± 6 kg) were included as controls. On the study day (13 ± 3 days after burn), a primed constant infusion (4 h) of stable isotope d_5-phenlylalanine and d_3-ketoisocaproic acid was given. Hourly arterial blood samples were drawn during the infusion to determine albumin synthesis rates, using gas chromatography–mass spectrometry analysis. Burned patients had higher heart and respiration rates. Plasma total protein in burn patients (4.5 ± 0.3 g · dL^{-1}) was lower compared with controls (6.8 ± 0.2 g · dL^{-1}). Plasma albumin concentration in burn patients (1.1 ± 0.1 g · dL^{-1}) was also lower compared with controls (3.8 ± 0.1 g · dL^{-1}; both $P < 0.05$). Albumin synthesis rate in burn patients (4.6 ± 0.2 mg · kg^{-1} · h^{-1}) was enhanced compared with controls (2.2 ± 0.2 mg · kg^{-1} · h^{-1}; $P < 0.05$). Despite the decrease in albumin concentration, albumin synthesis was enhanced in severely burned patients during the flow phase.

KEYWORDS—Burn injury, stable isotopes, gas chromatography and mass spectrometry

INTRODUCTION

Albumin is a predominant product of hepatic synthesis and is the most abundant plasma protein. The physiological functions of albumin include maintaining plasma osmotic pressure, binding and transporting free fatty acids, scavenging free radicals, and maintaining capillary membrane permeability (1). These important roles have made albumin concentration an important indicator of nutritional status, morbidity, and mortality in patients (2, 3).

Decreases in albumin levels have been commonly observed in patients during infection (4) and after trauma or surgery (5). Specifically after severe burn, plasma albumin concentration was universally observed to be decreased shortly after the injury and remained at depleted level even 8 weeks after the injury (6). Depletion may be due to inhibited synthesis, dilution, or increased disposal relative to synthesis before reaching steady state. To date, the mechanisms contributing to the decrease in plasma albumin concentrations remain unclear.

This study was designed to investigate changes in albumin synthesis in severely burned adult patients, using a stable isotope infusion technique with subsequent gas chromatography–mass spectrometry analysis. An age- and-sex-matched control group was included to compare the changes in albumin synthesis in relation to changes in albumin levels.

MATERIALS AND METHODS

Sterile stable isotope ring-d_5-phenylalanine (d_5-phenylalanine) and 5,5,5-d_3-ketoisocaproic acid (d_3-KIC, potassium salt) were purchased from Cambridge Isotope Laboratories (Andover, Mass). Sterile indocyanine green (ICG) dye and 0.45% normal saline were purchased from Akorn, Inc (Buffalo Grove, Ill) and Baxter Healthcare Corporation (Deerfield, Ill), respectively. Before study commencement, approval was obtained from the Brooke Army Medical Center institutional review board (H-04-017). Patients admitted to the burn unit at the US Army Institute of Surgical Research with severe burn equal to or greater than 20% total body surface area (TBSA) were screened. Those with preexisting chronic diseases (i.e., liver, heart, and lung), with acute life-threatening illness, who were pregnant or nursing, or with allergy to iodine or any dye were excluded. The isotope infusion study was performed on 13 ± 3 days after burn in enrolled patients while in a hypermetabolic state. Stable isotope solutions of d_5-phenylalanine (80 μmol · mL^{-1}) and d_3-KIC (120 μmol · mL^{-1}) were prepared at the Pharmacy Department at Brooke Army Medical Center under sterile and certified fume hoods. On the study day, after baseline physiological measurements and blood draws, primed constant infusions of d_5-phenylalanine (0.2 μmol · kg^{-1} · min^{-1}) and d_3-KIC (0.3 μmol · kg^{-1} · min^{-1}) were performed for 4 h in each subject through sterile 0.2-μm sterile and nonpyrogenic Tuffryn membrane filters (Paul Corporation, Ann Arbor, Mich). Albumin synthesis was measured based on the incorporation rate of phenylalanine tracer (d_5-phenylalanine) or leucine tracer (d_3-leucine) into the albumin molecule. The d_3-leucine was produced intracellularly from the infused d_3-KIC tracer. We considered that the plasma leucine enrichment would be closer to the precursor enrichment of leucine in the intracellular process of albumin synthesis. A similar method has been used to measure muscle protein synthesis rate (7). Hourly arterial blood samples were taken during the infusion. Indocyanine green dye was injected to measure plasma volumes during the infusion. Physiological measurements (i.e., heart rate, MAPs) were monitored and recorded hourly during the study.

Analytical methods

Blood chemistry was measured using a Dimension Clinical Chemistry System (Dade Behring Inc, Newark, Del), which included plasma total protein content and plasma concentrations of albumin and liver enzymes. Plasma

Address reprint requests to Wenjun Z. Martini, PhD, US Army Institute of Surgical Research, 3400 Rawley E. Chambers Ave, Ft Sam, Houston, TX 78234. E-mail: wenjun.martini@amedd.army.mil.

This study was supported by the US Army Medical Research and Materiel Command.

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

DOI: 10.1097/SHK.0b013e3181d8844b

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01 OCT 2010	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Enhanced albumin synthesis in severely burned adults	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
Martini W. Z., Wolf S. E., Chinkes D. L., Chung K. K., Dubick M. A., Blackbourne L., Yu Y. M.,	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)	8. PERFORMING ORGANIZATION REPORT NUMBER
United States Army Institute of Surgical Research, JBSA Fort Sam Houston, TX 78234	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**UU**	**5**	

TABLE 1. **Plasma concentrations of albumin, fibrinogen, and total protein in burned patients and normal volunteers (n = 5 per group)**

Groups	Albumin, $g \cdot dL^{-1}$	Fibrinogen, $mg \cdot dL^{-1}$	Total protein, $g \cdot dL^{-1}$
Control	3.8 ± 0.1	264.1 ± 30.9	6.8 ± 0.2
Burn	1.1 ± 0.2*	632.4 ± 41.9*	4.5 ± 0.3*

*$P < 0.05$ compared with control.

fibrinogen concentration was measured using the BCS Coagulation System (Dade Behring, Deerfield, Ill).

For assessment of plasma free amino acid enrichments, 0.5 mL of acidified plasma was loaded on a cation exchange column (AG 50W-X8 resin, 200–400 mesh, H^+ form; Bio-Rad, Hercules, Calif). Amino acids were separated after elution with ammonium hydroxide. The extracts were dried under speed vacuum and derivatized by *N*-methyl-*N*-(tert-butyldimethylsilyl)-trifluoroacetamide at 100°C for 1 h. Plasma albumin was isolated by ethanol extraction from trichloroacetic acid–precipitated plasma protein, following procedures described by Debro and Korner (8). The purity of the isolation has been documented previously (9, 10). The isolated albumin was dried and hydrolyzed in 6 N HCl at 110°C for 24 h. The released amino acids after hydrolysis were isolated, dried, and derivatized in the same manner as for plasma free amino acids. The enrichments of phenylalanine and leucine from the plasma free amino acid pool and from albumin protein were determined by gas chromatography–mass spectrometry analysis (model 5973; Hewlett-Packard, Palo Alto, Calif) in the electron impact ionization mode. A selective ion-monitoring method was used at nominal mass-to-charge ratio (*m/z*) of 336 ($m + 0$) and 341 ($m + 5$) for phenylalanine and 302 ($m + 0$) and 305 ($m + 3$) for leucine. The enrichment was expressed as tracer (labeled amino acids)–to–tracee (unlabeled amino acids) ratio (TTR).

Calculations

Plasma albumin fractional synthesis rate (FSR) was calculated using the convention formula (11):

$$FSR = (EB_{(t2)} - EB_{(t1)}) / (EF \times t)$$

where $EB_{(t)}$ is the enrichment of albumin-bound amino acids, and EF is the precursor from amino acid enrichment. In using tracer d_5-phenylalanine to calculate FSR, $EB_{(t)}$ was albumin-bound phenylalanine enrichment, and EF was plasma free phenylalanine enrichment at the steady state. In using tracer d_3-KIC to calculate FSR, $EB_{(t)}$ was albumin-bound leucine enrichment, and EF was the plasma free leucine enrichment at the steady state. Albumin half-life was calculated as ln2 / FSR.

The plasma albumin absolute synthesis rate (ASR) was calculated by multiplying FSR with plasma albumin concentration and plasma pool size, which was measured using ICG dye.

Statistical analysis

All results are expressed as mean ± SE. Comparisons in physiological measurements, liver enzyme activities, substrate concentrations, and albumin synthesis were made with two-sided nonpaired Student *t* test between the groups. Statistical significance was set at 0.05.

RESULTS

Following inclusion and exclusion criteria, five severely burned patients (29 ± 3 years old; 80 ± 7 kg; 48% ± 4% TBSA) consented to participate and were enrolled. All five enrolled patients received Peptinex DT (CWI Medical, Farmingdale, NY). Their nutritional needs were estimated at 3,386 ± 542 $kcal \cdot d^{-1}$ and 167 ± 24 g of protein (or 2.1 ± 0.4 $g \cdot kg^{-1}$ of protein per day). All enrolled patients received greater than 90% of the estimated needs based on tube feeds received, and none of them had active infection during the study. It is common practice in our ICU to use synthetic albumin as a maintenance and/or resuscitative fluid after 24 h of admission for up to 48 h. However, none of the enrolled patients received any exogenous albumin 48 h before the isotope infusion study. In addition, five age- and sex-matched normal healthy volunteers (33 ± 5 years old; 81 ± 6 kg) were studied as healthy controls.

All physiological measurements in the control and burn patients, respectively, remained unchanged during the isotope infusion, indicating a physiological steady state. Compared with the control group, burn patients had significant increases in respiration rate (25 ± 2 vs. 16 ± 2 breaths/min in control) and heart rate (114 ± 3 vs. 72 ± 7 beats/min in control; both $P < 0.05$). These signs indicated a severe burn–induced hypermetabolic and hyperhemodynamic status (12). Burned subjects had decreased levels of plasma albumin and total protein and increased levels of plasma fibrinogen (Table 1). Plasma volumes, measured by ICG dye, were higher in burn patients (50 ± 2 $mL \cdot kg^{-1}$) compared with control values (35 ± 2 $mL \cdot kg^{-1}$; $P < 0.05$). Serum liver enzyme concentrations in burn patients were significantly higher than those in the control group ($P < 0.05$) (Table 2).

The enrichments of plasma free phenylalanine TTR ($m + 5$) from d_5-phenylalanine infusion in both groups reached plateau values (29.5% ± 2.0% in control and 15.0% ± 1.3% in burn patients) after 1 h of d_5-phenylalanine infusion. Similarly, the enrichments of plasma free leucine TTR (m + 3) from d_3-KIC infusion in both groups reached plateau values (13.2% ± 1.0% in control and 5.3% ± 0.6%) in burn patients after 1 h of d_3-KIC infusion. The calculated appearance rate of free phenylalanine, which is a reflection of whole-body protein breakdown, was 0.76 ± 0.04 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in control and 1.80 ± 0.18 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in burn patients ($P < 0.05$). The appearance rate of free leucine was 2.07 ± 0.14 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in control and 6.00 ± 0.71 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in burn patients ($P < 0.05$), indicating a severe protein catabolic state.

The enrichments of albumin-bound phenylalanine TTR ($m + 5$) showed a linear increase during the infusion of d_5-phenylalanine. Plasma albumin FSR was calculated from the increases in albumin enrichments during the 4-h-infusion study. Albumin FSR measured by d_5-phenylalnine was 0.17% ± 0.01% per hour in control and 0.86% ± 0.11% per hour in burn patients ($P < 0.05$). Albumin ASR, calculated by multiplying albumin FSR with albumin concentrations and

TABLE 2. **Serum liver enzyme concentrations in burned patients and normal volunteers (n = 5 per group)**

Groups	Aspartate aminotransferase, $U \cdot L^{-1}$	Alanine aminotransferase, $U \cdot L^{-1}$	Lactate dehydrogenase, $U \cdot L^{-1}$	γ-Glutamyl transferase, $U \cdot L^{-1}$
Control	17 ± 3	33 ± 4	145 ± 8	21 ± 4
Burn	99 ± 27*	119 ± 26*	272 ± 34*	103 ± 36*

*$P < 0.05$ compared with control.

plasma volumes, was 2.2 ± 0.2 mg · kg^{-1} · h^{-1} in control and 4.62 ± 0.24 mg · kg^{-1} · h^{-1} in burn patients by tracer d_5-phyenlalanine ($P < 0.05$). The half-life of albumin decreased from control value of 17 ± 1 to 4 ± 1 days in burn patients ($P < 0.05$), indicating albumin turnover was accelerated by burn injury.

Similarly, the enrichments of albumin-bound leucine TTR (m + 3) showed a linear increase during the infusion d_3-KIC. Plasma albumin FSR measured by d_3-KIC was 0.21% ± 0.02% per hour in control and 0.85% ± 0.09% per hour in burn patients ($P < 0.05$). Albumin ASR was 2.7 ± 0.2 mg · kg^{-1} · h^{-1} in control and 4.63 ± 0.18 mg · kg^{-1} · h^{-1} in burn patients by tracer d_3-KIC ($P < 0.05$). The half-life of albumin decreased from control value of 16 ± 1 to 5 ± 1 days in burn patients ($P < 0.05$). It is worth mentioning that, within each group, the values of FSR and ASR measured by the two tracers (d_5-phenylalanine and d_3-KIC) were similar, and significant increases in FSR and ASR by severe burn were shown by both tracers independently.

DISCUSSION

Albumin plays important roles in host homeostasis. Changes in albumin synthesis have been investigated in patients with various diseases, such as cirrhosis, uremia, and diabetes (9, 13, 14). Decreased albumin concentration has been widely recognized as an acute-phase response following burn injury. In this study, we investigated the contributions of albumin synthesis to changes in albumin concentration in severely burned adults. Our data demonstrate that despite the decrease in plasma albumin concentration, albumin endogenous synthesis was highly accelerated following severe burn. Thus, hypoalbuminemia observed in burn patients does not seem to be secondary to inhibited synthesis.

The important roles of albumin have initiated efforts in investigating albumin kinetics. As the most abundant plasma protein, albumin is exclusively synthesized in the liver. In normal subjects, albumin synthesis is about 5% to 10% per day, and the half-life of albumin is 16 to 19 days (15–19). Under normal conditions, albumin disposal takes place primarily in the vascular endothelium (20), and the catabolic rate is approximately 10% per day, similar to that of synthesis (18). Although decreased albumin levels are commonly observed in burned patients, limited information exists on the effects of injury on albumin metabolism. In this study, using stable isotopic techniques, we observed a 100% increase in albumin synthesis in severely burned adults, although the albumin concentration in these patients was less than 30% of the control values. This observation demonstrates that the depletion of albumin concentration was not likely due to insufficient albumin synthesis. In addition, we did not observe a correlation of changes in albumin synthesis with burn size in this study. This is possibly due to the severity of burn in this study. The TBSA in the enrolled patients was 48% ± 4% in this study, and protein catabolism in burn patients has been shown to plateau at 40% TBSA (21).

It is worth mentioning that even with the 100% increase in albumin synthesis, albumin synthesis rate remains to be relatively small compared with its pool size (<1% of the total albumin pool size was synthesized per hour). Thus, it may take days for changes in synthesis to be reflected in albumin levels, if other kinetic parameters remain unchanged. In other words, changes in albumin synthesis are unlikely to have a prompt and significant impact on albumin concentrations.

As a transport protein, albumin has a molecular weight of 65,000, and about 50% of its content is confined in the vascular pool. In contrast to albumin synthesis rate, albumin transport through the transcapillary membrane is very rapid. In the past, albumin transport was assessed as the transvascular escape rate (TER), an estimate of the loss of albumin across the vascular endothelium based on intravenous injection of radioactive-labeled albumin. Transvascular escape rate of albumin in normal subjects is found to be about 5% per hour (5, 18, 22), which is more than 20 times higher than that of normal albumin synthesis. Thus, changes in albumin transport are likely to have a significant impact on plasma albumin levels. It is also likely that some albumin lost to the intravascular space will return in a "give and take"; thus, concentration values remain unchanged under normal conditions. Following surgical trauma, Fleck et al. (5) reported a 2-fold increase in TER within a few hours after the surgery. Transvascular escape rate was also shown to have increased in patients with a combination of hypoalbuminemia and cancer (23, 24), infection (25), and hypertension (22). After severe burn, vascular permeability is increased following injury (26–28), and significant changes in vascular permeability on plasma fluids and osmotically active proteins occur within hours (29). Substances with a molecular weight of 125,000 are found to pass freely through the injured capillary membrane (30). In addition, capillary permeability was also found to be increased even at unburned areas (31). Because albumin TER is already high under normal circumstances, the increase in vascular permeability by burn may further amplify albumin TER. Furthermore, the balanced give-and-take under normal conditions may be disturbed by burn, with possibly increased leakage to the extravascular pool and decreased return to the intravascular pool. Therefore, the decrease in plasma albumin levels may likely be due to the increase in albumin leak to the extravascular pool.

Protein is synthesized from amino acids and is regulated by the availability of intracellular amino acids. Metabolic responses to stress of trauma, inflammation, or infection are generalized by an increase in whole-body protein turnover rate, with a net loss of body protein (32, 33). Amino acid release from muscle and amino acid uptake in the splanchnic bed are increased (33). The shift of amino acid sources from muscle to liver is considered to be beneficial as it facilitates hepatic synthesis of proteins, which are critical for survival. Similar findings were observed in this study. Following severe burn, we observed an increase in whole-body protein breakdown as reflected by the increases in appearance rates of phenylalanine and leucine, as well as increases in hepatic synthesis of albumin. Thus, the increase in albumin synthesis in this study may result from the increase in intracellular amino acid availability from accelerated whole-body protein breakdown. It should be emphasized that albumin synthesis

is a dynamic process, and data presented in this study are only a glimpse of albumin synthesis during the flow phase of recovery. Changes in albumin synthesis at different periods, such as shortly after burn or during the outpatient convalescent phase, may differ. In fact, a biphase pattern of albumin synthesis has been suggested from previous animal studies. When intra-abdominal sepsis was induced in rats, albumin synthesis was found to be unchanged at 16 h (34) but increased by 74% at 96 h after the insult (35). Future investigation will clarify whether the biphase pattern of albumin synthesis is also present in burned patients.

Albumin infusion has been in clinical practice for more than 50 years. It is widely used in hypoalbuminemic burn patients to raise plasma albumin concentrations. Because vascular permeability and albumin leak to the extravascular pool are increased by injury, caution is advised in administering exogenous albumin, especially because the effects of albumin leaking to the extravascular pool are not clear. In addition, albumin concentration has been used as an indicator of nutritional status, although good correlation of plasma albumin level and nutritional status is lacking (36–38). Data from this study demonstrate that hepatic albumin synthesis was not compromised in burn patients, which therefore questions the role of infusing exogenous amino acids to stimulate albumin synthesis to correct plasma albumin concentration.

In tracer methodology, protein synthesis is quantified by the rate of tracer (amino acids) incorporation to protein over time divided by the precursor enrichments (11). Because the charged tRNAs are assembled along the ribosome according to the codons of the mRNA during protein synthesis, the enrichments of tRNA-bound amino acid likely represent true precursor enrichments for the protein synthesis calculation. However, accurate measurement of tRNA enrichment is difficult. Consequently, different precursor surrogates for precursor enrichment have been used. The enrichment of plasma free amino acid is an easier measurement, but often provides an underestimation of the true intracellular enrichment because of the intracellular dilution by the unlabeled amino acid released from intracellular protein breakdown. However, phenylalanine has been shown to be an exception (39). When tracer KIC is infused, it is converted to leucine intracellularly and released to the bloodstream. Thus, plasma enrichment of leucine from KIC infusion more closely represents intracellular enrichments. In this study, both approaches (plasma phenylalanine enrichments from d_5-phenylalanine infusion and leucine enrichments from d_3-KIC infusion) were used to quantify albumin synthesis. We observed comparable results of albumin synthesis from both independent tracers within the normal group and the burn group, as well as consistent conclusions regarding changes in albumin synthesis by burn in this study. Thus, it is reasonable to consider that our quantification of albumin synthesis in this study is acceptable.

In conclusion, albumin synthesis was increased in severely burned patients at 13 days after injury. The increase in albumin synthesis, however, did not correct albumin concentrations, which was much lower those in matched control subjects. Further investigation is required to reveal the role of albumin disposal to albumin concentrations to determine the best treatment options in injured patients with depleted albumin levels.

ACKNOWLEDGMENTS

The authors thank Annette McClinton for her regulatory assistance throughout the study. The authors appreciate the support received from the research nurses at the US Army Institute of Surgical Research and the Pharmacy Department at Brooke Army Medical Center.

REFERENCES

1. Chen LW, Wang JS, Hwang B, Chen JS, Hsu CM: Reversal of the effect of albumin on gut barrier function in burn by the inhibition of inducible isoform of nitric oxide synthase. *Arch Surg* 138:1219–1225, 2003.
2. Gibbs J, Cull W, Henderson W, Daley J, Hur K, Khuri SF: Preoperative serum albumin level as a predictor of operative mortality and morbidity: results from the National VA Surgical Risk Study. *Arch Surg* 134:36–42, 1999.
3. Vincent JL, Navickis RJ, Wilkes MM: Morbidity in hospitalized patients receiving human albumin: a meta-analysis of randomized, controlled trials. *Crit Care Med* 32:2029–2038, 2004.
4. Belfrage S: Plasma protein pattern in course of acute infectious disease. *Acta Med Scand* 395:1–169, 1963.
5. Fleck A, Raines G, Hawker F, Trotter J, Wallace PI, Ledingham IM, Calman KC: Increased vascular permeability: a major cause of hypoalbuminaemia in disease and injury. *Lancet* 1:781–784, 1985.
6. Bonate PL: Pathophysiology and pharmacokinetics following burn injury. *Clin Pharmacokinet* 18:118–130, 1990.
7. Chinkes DL, Rosenblatt J, Wolfe RR: Assessment of the mathematical issues involved in measuring the fractional synthesis rate of protein using the flooding dose technique. *Clin Sci (Lond)* 84:177–183, 1993.
8. Debro JR, Korner A: Solubility of albumin in alcohol after precipitation by trichloroacetic acid: a simplified procedure for separation of albumin. *Nature* 178:1067, 1956.
9. Ballmer PE, Walshe D, McNurlan MA, Watson H, Brunt PW, Garlick PJ: Albumin synthesis rates in cirrhosis: correlation with Child-Turcotte classification. *Hepatology (Baltimore, Md)* 18:292–297, 1993.
10. Hunter KA, Garlick PJ, Broom I, Anderson SE, McNurlan MA: Effects of smoking and abstention from smoking on fibrinogen synthesis in humans. *Clin Sci (Lond)* 100:459–465, 2001.
11. Wolfe RR, Chinkes DL: *Isotope Tracers in Metabolic Research: Principles and Practice of Kinetic Analysis*. 2nd ed. Hoboken, NJ: Jon WIley & Sons, Inc, 2005.
12. Yang E, Maguire T, Yarmush ML, Berthiaume F, Androulakis IP: Bioinformatics analysis of the early inflammatory response in a rat thermal injury model. *BMC Bioinform* 8:10, 2007.
13. Demant T, Mathes C, Gutlich K, Bedynek A, Steinhauer HB, Bosch T, Packard CJ, Warwick GL: A simultaneous study of the metabolism of apolipoprotein b and albumin in nephrotic patients. *Kidney Int* 54:2064–2080, 1998.
14. Sironi AM, Vichi S, Gastaldelli A, Pecori N, Anichini R, Foot E, Seghieri G, Ferrannini E: Effects of troglitazone on insulin action and cardiovascular risk factors in patients with non-insulin-dependent diabetes. *Clin Pharmacol Ther* 62:194–202, 1997.
15. Mansoor O, Cayol M, Gachon P, Boirie Y, Schoeffler P, Obled C, Beaufrere B: Albumin and fibrinogen syntheses increase while muscle protein synthesis decreases in head-injured patients. *Am J Physiol* 273:E898–E902, 1997.
16. Jackson AA, Phillips G, McClelland I, Jahoor F: Synthesis of hepatic secretory proteins in normal adults consuming a diet marginally adequate in protein. *Am J Physiol* 281:G1179–G1187, 2001.
17. Imoberdorf R, Garlick PJ, McNurlan MA, Casella GA, Peheim E, Turgay M, Bartsch P, Ballmer PE: Enhanced synthesis of albumin and fibrinogen at high altitude. *J Appl Physiol* 90:528–537, 2001.
18. Fleck A: Plasma proteins as nutritional indicators in the perioperative period. *Br J Clin Pract* 63:20–24, 1988.
19. De Feo P, Volpi E, Lucidi P, Cruciani G, Reboldi G, Siepi D, Mannarino E, Santeusanio F, Brunetti P, Bolli GB: Physiological increments in plasma insulin concentrations have selective and different effects on synthesis of hepatic proteins in normal humans. *Diabetes* 42:995–1002, 1993.
20. Mendez CM, McClain CJ, Marsano LS: Albumin therapy in clinical practice. *Nutr Clin Pract* 20:314–320, 2005.
21. Hart DW, Wolf SE, Chinkes DL, Gore DC, Mlcak RP, Beauford RB, Obeng MK, Lal S, Gold WF, Wolfe RR, et al.: Determinants of skeletal muscle catabolism after severe burn. *Ann Surg* 232:455–465, 2000.
22. Parving HP, Gyntelberg F: Transcapillary escape rate of albumin and plasma volume in essential hypertension. *Circ res* 32:643–651, 1973.

23. de Vries EG, Beekhuis H, de Jong R, Mulder NH, Piers DA: Evidence for capillary leakage during chemotherapy in man. *Eur J Clin Invest* 16:243–247, 1986.
24. Ballmer PE, Ochsenbein AF, Schutz-Hofmann S: Transcapillary escape rate of albumin positively correlates with plasma albumin concentration in acute but not in chronic inflammatory disease. *Metabolism* 43:697–705, 1994.
25. Grossman J, Yalow AA, Weston RE: Albumin degradation and synthesis as influenced by hydrocortisone, corticotropin and infection. *Metabolism* 9:528–550, 1960.
26. Ryan CM, Yarmush ML, Burke JF, Tompkins RG: Increased gut permeability early after burns correlates with the extent of burn injury. *Crit Care Med* 20:1508–1512, 1992.
27. Order SE, Moncrief JA: Vascular destruction and revascularization in severe thermal injuries. *Surg Forum* 15:37–39, 1964.
28. LeVoyer T, Cioffi WG Jr, Pratt L, Shippee R, McManus WF, Mason AD Jr, Pruitt BA Jr: Alterations in intestinal permeability after thermal injury. *Arch Surg* 127:26–29; discussion 29–30, 1992.
29. Cope O, Moore FD: A study of capillary permeability in experimental burns and burn shock using radioactive dyes in blood and lymph. *J Clin Invest* 23:241–257, 1944.
30. Ricketts CR: Molecular permeability of the burned area. *Proc Soc Exp Biol Med (New York, NY)* 121:770–772, 1966.
31. Arturson G: Pathophysiological aspects of the burn syndrome with special reference to liver injury and alterations of capillary permeability. *Acta Chir Scand Suppl* 274:1–135, 1961.
32. Jahoor F, Desai M, Herndon DN, Wolfe RR: Dynamics of the protein metabolic response to burn injury. *Metabolism* 37:330–337, 1988.
33. Clowes GH Jr, Randall HT, Cha CJ: Amino acid and energy metabolism in septic and traumatized patients. *JPEN* 4:195–205, 1980.
34. Sax HC, Talamini MA, Hasselgren PO, Rosenblum L, Ogle CK, Fischer JE: Increased synthesis of secreted hepatic proteins during abdominal sepsis. *J Surg Res* 44:109–116, 1988.
35. von Allmen D, Hasselgren PO, Fischer JE: Hepatic protein synthesis in a modified septic rat model. *J Surg Res* 48:476–480, 1990.
36. Waitzberg DL, Correia MI: Nutritional assessment in the hospitalized patient. *Curr Opin Clin Nutr Metab Care* 6:531–538, 2003.
37. Fuhrman MP: The albumin-nutrition connection: separating myth from fact. *Nutrition (Burbank, Los Angeles County, Calif)* 18:199–200, 2002.
38. Downs JH, Haffejee A: Nutritional assessment in the critically ill. *Curr Opin Clin Nutr Metab Care* 1:275–279, 1998.
39. Martini WZ, Chinkes DL, Wolfe RR: The intracellular free amino acid pool represents tracer precursor enrichment for calculation of protein synthesis in cultured fibroblasts and myocytes. *J Nutr* 134:1546–1550, 2004.